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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY AND
POLLUTION PREVENTION

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

MEMORANDUM

Date: March 16, 2011

SUBJECT: Saflufenacil immuotoxicity study

PC Code: 118203
Decision No.: 441331
Petition No.: N/A
Risk Assessment Type: N/A
TXR No.: 0055710
MRID No.: 48233701

DP Barcode: D383475
Registration No.: 7969-275
Regulatory Action: N/A
Submission No.: 883830
CAS No.: 372137-35-4
40 CFR: N/A

FROM: **Ayaad Assaad, D.V.M., Ph.D.**
RAB IV,
Health Effects Division (7509 P)
and
Yung Yang, Ph.D.
RAB VI,
Health Effects Division (7509 P)

Ayaad Assaad 3/16/2011

Yung G. Yang

THROUGH: **Deborah Smegal, Acting Chief**
RAB IV,
Health Effects Division (7509 P)

TO: **James Tompkins**
FB, RM 25
Registration Division (7505 P)
and
Dana Vogel, Chief
RAB I
Health Effects Division (7509P)

I. CONCLUSIONS:

RAB IV has reviewed the immunotoxicity study for Saflufenacil (MRID # 48233701) and concluded that this study is classified as acceptable/guideline and satisfies the guideline requirement for an immunotoxicity study (OPPTS 870.7800).

Received in HRC 3/16/2011

II. BACKGROUND and ACTION REQUESTED

The registrant, BASF Corporation, Agricultural Products has submitted this immunotoxicity study with Saflufenacil in mice. RAB I requested RAB IV to review and prepare DER for this study. The DER is attached and an executive summary is as follows.

EXECUTIVE SUMMARY:

In an immunotoxicity study (MRID # 48233701), Saflufenacil (93.8% a.i., batch # COD-000515) was administered to 8 male C57BL/6J Rj mice in diet at dose levels of 0, 50, 125, or 250 ppm (0, 10, 27, or 52 mg/kg/day) over a period of 4 weeks. Additional 8 male C57BL/6 J Rj mice received Cyclophosphamide monohydrate at a dose level of 10 mg/kg/day by gavage for 4 weeks (positive control group). The male mouse is the appropriate species/sex for this study. Parameters evaluated were food consumption and body weight (once a week), signs of toxicity or mortality at least once a day. All animals were assessed by gross pathology and liver, spleen and thymus weights were measured. In addition, the livers of all animals in control and 250 ppm test groups were examined histopathologically. In addition, limited hematological and clinical chemistry parameters were evaluated.

On Day 23, all animals were immunized intraperitoneally with 0.5 mL of sheep red blood cells (4×10^8 SRBC/mL). On Day 29, the immunotoxic potential of saflufenacil was assessed through the evaluation of the T-cell dependent antibody response (TDAR) to sheep red blood cells (SRBC) using an enzyme-linked immunsorbent assay (ELISA).

Saflufenacil did not cause mortality or any signs of general toxicity in any treated group; body weights and body weight changes, food consumption were not affected by treatment. Absolute and relative liver weights at the highest dose were significantly increased and alanine aminotransferase (ALT) activity was also increased. Histopathological evaluation of the liver revealed a slight to moderate centrilobular fatty change in almost all animals (7 out of 8) of the high dose group. Additionally, two animals showed minimal lymphoid infiltration and a single animal showed minimal extramedullary hematopoiesis in the liver. Red blood cell (RBC) counts, hemoglobin and hematocrit values were decreased in both mid and high dose groups. Cyclophosphamide treated animals showed significant decreases of terminal body weights, thymus and spleen weights (relative weights were not significant), and RBC, hemoglobin and hematocrit values, while mean corpuscular volume (MCV) and the mean corpuscular hemoglobin content (MCH) were increased.

The systemic NOAEL is 125 ppm (27 mg/kg/day), the LOAEL was 250 ppm (52 mg/kg/day) based on increased liver weights, ALT level and histopathologic findings in the liver.

There were no treatment-related effects in the anti-SRBC IgM titers in mice dosed with saflufenacil. Evaluation of individual animal data of the treatment and control groups did not show any trend or distribution that would demonstrate a significant suppression of

anti-SRBC IgM response. Positive control group demonstrated significant suppression of anti-SRBC IgM response compared with the control group.

The NK cells activity assay was not performed. Evaluation of toxicity database of saflufenacil identified the hematopoietic system as the target organ. Increased spleen weights were seen in rat study which was attributable to an increased clearance of defective RBCs (i.e, defective hemoglobin synthesis) and is thus an indication of toxicity to the hematopoietic system rather than to the immune system. The overall weight of evidence suggests that this chemical does not directly target the immune system. Under the HED guidance, if the TDAR assay is negative and evaluation of observational endpoints from all available toxicology database provide no evidence of immunotoxicity, the test article is considered no evidence of immunotoxicity and evaluation of NK cells activity is not necessary.

Under conditions of this study, the NOAEL for anti-SRBC IgM response is 250 ppm (52 mg/kg body weight/day). The LOAEL was not established.

This immunotoxicity study is classified acceptable/guideline and satisfies the guideline requirement for an immunotoxicity study (OPPTS 870.7800) in mice.

EPA Reviewer: Ayaad Assaad, D.V.M., Ph.D.
RAB IV, Health Effects Division (7509P)
EPA Secondary Reviewer: Yung G. Yang, Ph.D.
RAB VI, Health Effects Division (7509P)

Signature: A Assaad
Date: 3/16/2011
Signature: Yung G. Yang
Date: 3/16/2011

Template version 02/06

TXR #: 0055710

DATA EVALUATION RECORD

STUDY TYPE: Immunotoxicity [feeding] Study in mice OPPTS 870.7800

PC CODE: 118203 **DP BARCODE:** 383475

TEST MATERIAL (PURITY): Saflufenacil (93.8%, a.i.)

SYNONYMS: BAS 800 H

CITATION: R. Buesen, V. Strauss, S. Groeters (2010), BAS 800 H (Saflufenacil) - Immunotoxicity study in male C57BL/6 J Rj mice-Administration in the diet for 4 weeks. Laboratory name: Experimental Toxicology and Ecology BASF (SE 67056 Ludwigshafen, Germany). Laboratory report number: 43C0414/01S002, full study date: October 06, 2010. MRID # 48233701. Unpublished.

SPONSOR: BASF Corporation, Agricultural Products.

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On Day 23, all animals were immunized intraperitoneally with 0.5 mL of sheep red blood cells (4×10^8 SRBC/mL). On Day 29, the immunotoxic potential of saflufenacil was assessed through the evaluation of the T-cell dependent antibody response (TDAR) to sheep red blood cells (SRBC) using an enzyme-linked immunsorbent assay (ELISA).

Saflufenacil did not cause mortality or any signs of general toxicity in any treated group; body weights and body weight changes, food consumption were not affected by treatment. Absolute and relative liver weights at the highest dose were significantly increased and alanine aminotransferase (ALT) activity was also increased. Histopathological evaluation of the liver

revealed a slight to moderate centrilobular fatty change in almost all animals (7 out of 8) of the high dose group. Additionally, two animals showed minimal lymphoid infiltration and a single animal showed minimal extramedullary hematopoiesis in the liver. Red blood cell (RBC) counts, hemoglobin and hematocrit values were decreased in both mid and high dose groups. Cyclophosphamide treated animals showed significant decreases of terminal body weights, thymus and spleen weights (relative weights were not significant), and RBC, hemoglobin and hematocrit values, while mean corpuscular volume (MCV) and the mean corpuscular hemoglobin content (MCH) were increased.

The systemic NOAEL is 125 ppm (27 mg/kg/day), the LOAEL was 250 ppm (52 mg/kg/day) based on increased liver weights, ALT level and histopathologic findings in the liver.

There were no treatment-related effects in the anti-SRBC IgM titers in mice dosed with saflufenacil. Evaluation of individual animal data of the treatment and control groups did not show any trend or distribution that would demonstrate a significant suppression of anti-SRBC IgM response. Positive control group demonstrated significant suppression of anti-SRBC IgM response compared with the control group.

The NK cells activity assay was not performed. Evaluation of toxicity database of saflufenacil identified the hematopoietic system as the target organ. Increased spleen weights were seen in rat study which was attributable to an increased clearance of defective RBCs (i.e, defective hemoglobin synthesis) and is thus an indication of toxicity to the hematopoietic system rather than to the immune system. The overall weight of evidence suggests that this chemical does not directly target the immune system. Under the HED guidance, if the TDAR assay is negative and evaluation of observational endpoints from all available toxicology database provide no evidence of immunotoxicity, the test article is considered no evidence of immunotoxicity and evaluation of NK cells activity is not necessary.

Under conditions of this study, the NOAEL for anti-SRBC IgM response is 250 ppm (52 mg/kg body weight/day). The LOAEL was not established.

This immunotoxicity study is classified acceptable/guideline and satisfies the guideline requirement for an immunotoxicity study (OPPTS 870.7800) in mice.

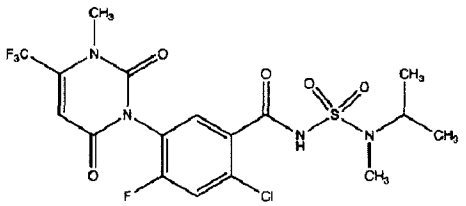
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: Saflufenacil

Description:	Technical, solid/light beige
Lot/Batch #:	COD-000515
Purity:	93.8 % a.i.
Compound Stability:	Stable at room temperature for 49 days.
CAS # of TGAI:	372137-35-4
Chemical structure:	



2. Vehicle and/or positive control: Ground Kliba maintenance diet mouse/rat "GLP" meal.

3. Positive control: Cyclophosphamide monohydrate (SIGMA-ALDRICH, Taufkirchen, Germany), CAS # 6055-19-2.

4. Test animals:

Species:	Mouse	
Strain:	Male C57BL/6 J Rj	
Age/weight at study initiation:	49±1 days	
Source:	Raison sociale, France	
Housing:	The mice were housed individually in Polycarbonate cages, type M II with wire cover. Bedding in the cages were Type Lignocel FS 14, dust-free bedding.	
Diet:	Ground Kliba maintenance diet mouse/rat "GLP", meal *ad libitum*	
Water:	Municipal drinking water *ad libitum*	
Environmental conditions:	**Temperature:**	20 - 24°C
	Humidity:	30-70%
	Air changes:	Not reported
	Photoperiod:	12 hrs dark/ 12hrs light
Acclimation period:	Six days	

B. STUDY DESIGN:

1. In life dates - Start: February 22/2010 End: March 23/2010

2. Animal selection and assignment: There was a sex-dependent difference in the excretion of orally administered saflufenacil. The male mouse is the appropriate species/sex for this immunotoxicity study based on toxicity database (see dose selection section for details). Prior to the first detailed clinical observation, the animals were distributed according to weight among the individual test groups. The weight variation of the animals used did not exceed 20 percent of the mean weight. The list of randomization instructions was compiled with a computer, and animals were assigned to the test groups noted in Table 1.

TABLE 1: Study design[a]				
Test group	**Conc. in diet (PPM)**	**Dose to animal Mg/kg/day**	**# Male**	**# Female**
Control	0	0	8	0
Low	50	10	8	0
Mid	125	27	8	0
High	250	52	8	0
Positive control[b] (Cyclophosphamide)	N/A	10 mg/kg (gavage)	8	0

[a]Data obtained from page 23, MRID # 48233701

3. Dose selection

The dose selection was based on 28-day and 90-day oral toxicity studies in mice where the LOAEL was 150 ppm (36.7mg/kg bw/d) in males based on multiple hematological changes, liver weight increases with centrilobular fatty change and lymphoid infiltrate in males. The NOAEL was 50 ppm (12.5mg/kg bw/d). In females, the LOAEL was 450 ppm (156.6 mg/kg/d) based on increased liver weight with centrilobular fatty change and lymphoid infiltrate. The NOAEL was 150 ppm (51.8 mg/kg/d).

4. Diet preparation and analysis

For each concentration, the test substance (BAS 800 H [Saflufenacil]) was weighed out and mixed with a small amount of food (Ground Kliba maintenance). Then corresponding amounts of food, depending on the test group, were added to this premix in order to obtain the desired concentrations. Mixing was carried out for about 10 minutes in a laboratory mixer. The test-substance preparations were mixed once before the start of the administration period and stored at room temperature. The stability of the test substance BAS 800 H (Saflufenacil) in the diet was demonstrated over a period of 49 days at room temperature. As the mixtures were stored no longer than this time period, the stability was guaranteed. Concentrations of the test substance in the diet were always in a range of 91.0% - 93.3%.

Results

Homogeneity analysis: The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable. Target test substance concentrations of 50 and 250 ppm Saflufenacil met the requirement for homogeneity (Table. 2).

TABLE 2: Concentration and homogeneity analysis of Saflufenacil [a]			
Test group	**Conc. in diet (ppm)**	**Dose to animal (mg/kg/day)**	**% of Target conc.**
Control	0	0	0
Low	50	10	92.4±4.4
Mid	125	27	93.3±2.5
High	250	52	91±0.8

[a]Data obtained from page 157, MRID # 48233701

Stability analysis: Saflufenacil was stable in feed for 49 days of at room temperature storage. Stability ranged between 102.7-97.9%.

Concentration analysis: Analyzed formulations (0, 50, 125, and 250 ppm Saflufenacil used for test substance administration were within 91% to 93% of the target concentration.

5. **Statistics:** Statistical analysis is detailed in Table 3.

TABLE 3: Statistical analysis of Saflufenacil data[a]

Parameter(s)	Statistical method
Food consumption, body weight, and body weight change.	-Two-sided DUNNETT's test to compare each group with the control (groups 0, 1, 2, 3). -Two-sided *t* test for groups 0 vs. 4.
-Clinical pathology parameters. -Immunotoxicological parameters.	-Non-parametric one-way analysis using KRUSKAL-WALLIS test (groups 0, 1, 2, 3). -Pair-wise comparison with the control group using WILCOXON test (two-sided) for groups 0 vs. 4.
Organ weights.	-Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided).
Statistically significant level of ($p < 0.05$) was used to report data	

[a]Data obtained from pages 28, 32, 34, MRID # 48233701

C. METHODS:

1. Observations:

All animals were checked daily for any clinically abnormal signs. Detailed clinical observations (DCO) were performed in all animals prior to the administration period and thereafter at weekly intervals. A check for moribund and dead animals was made twice daily on working days and once a day during weekends.

2. Body weight:

Body weight was determined before the start of the administration period, on day 0 (start of the administration), and thereafter at weekly intervals.

3. Food/water consumption and compound intake:

Individual food consumption was determined once a week and calculated as mean food consumption in grams per animal/day. Compound intake (mg/kg bw/day) values were calculated as time-weighted averages from the consumption and body weight gain data:

$\frac{FC_x \times C}{BW_x}$ = test-substance intake for day x

Where:
BW_x = body weight on day x [g]
FC_x = mean daily food consumption on day x [g]
C = concentration in the food on day x [mg/kg]

4. Sacrifice and pathology

Animals were sacrificed on day 29 by decapitation under isoflurane anesthesia after fasting period of at least 16-20 hours.

a. Gross necropsy:

The exsanguinated animals were necropsized and assessed grossly for potential gross pathological changes. Weight assessment was carried out on all animals sacrificed at scheduled dates. The following weights were determined: anesthetized animals, liver, spleen, and thymus.

b. Tissue preparation/histopathology:

The following organs or tissues were fixed in 4% buffered formaldehyde solution: all gross lesions, liver, spleen, and thymus. Liver sections were fixed in Carnoy's solution and embedded in paraplas. Fixation was followed by histotechnical processing, examination by light microscopy, and assessment of findings.

5. CLINICAL PATHOLOGY:

In the morning, blood was taken from the retro-orbital venous plexus for hematology, and after decapitation for serum chemistry from fasted animals. The animals were anaesthetized with isoflurane.

6. Immunotoxicity:

a. Enzyme-Linked Immunosorbent Assay (ELISA):

The immunotoxic potential of Saflufenacil was assessed through the evaluation of the primary antibody response to sheep red blood cells (SRBC) using an enzyme-linked immunosorbent assay (ELISA) approach that measured the concentration of serum anti-SRBC IgM. Six days prior to sacrifice, mice were immunized with a single, 0.5 ml i.p. injection of SRBC (4×10^8 SRBC/mL). Blood samples for measuring anti-SRBC IgM were obtained from the orbital sinus. Serum samples were analyzed for anti-SRBC IgM using a commercially available ELISA kit, according to manufacturer's recommendations with some modifications. Optical density was measured spectrophotometrically. The concentration of anti-SRBC IgM was proportional to the optical density of the test sample. Concentration of the anti-SRBC IgM in the test samples was measured by a standard curve prepared by serial dilution of mouse anti-SRBC standard stock.

b. Natural Killer (NK) cells activity assay: Not performed.

II. RESULTS:

A. OBSERVATIONs:

1. Clinical signs of toxicity:

The test substance BAS 800 H (Saflufenacil) did not cause any signs of general systemic toxicity in any test group. Four animals in the positive control (cyclophosphamide) group showed moderate to severe reduced general condition from Day 23 onward until the end of the study.

2. Mortality:

No animal died prematurely in the present study.

B. Body weight and weight gain: Body weight as well as body weight gains of all animals treated with BAS 800 H (Saflufenacil) were not affected by test substance administration. Body weight and body weight change of mice treated with Cyclophosphamide monohydrate (positive control group) were impaired throughout the whole administration period, reaching a maximum on study day 28, i.e. -10.99% (mean body weight) and -67.20% (mean body weight changes) (Table 4).

TABLE 4. Average body weights and body weight gains during 28 days of treatment

Dose rate (ppm)	Body weights (g±SD)					Total weight gain	
	Day 0	Day 7	Day 14	Day 21	Day 28	g	% Control
Male							
0 (n=8)	19.8±0.8	21.4±1	22.1±1	22.9±1.5	23.7±1	3.9±0.9	--
Low (n=8)	19.9±0.8	21.6±0.8	22.5±1	23.5±1	23.7±1	3.8±1.2	97%
Mid (n=8)	19.8±0.8	21.3±0.7	22±0.8	22.7±0.9	23.2±1	3.4±0.5	87%
High (n=8)	20±1	21.7±0.9	22.9±1	23.8±1	24.3±1	4.3±1.2	110%
Cyclophosphamide (n=8)	19.8±07	20.7±0.9	21.1±0.7	21.2±1.8	21.1±2.5*	1.3±2.6	33%**

[a] Data obtained from pages 52 & 53 , MRID # 48233701.
* Statistically different ($p < 0.05$) from the control.
** Statistically different ($p < 0.01$) from the control.

C. FOOD/WATER CONSUMPTION AND COMPOUND INTAKE:

1. **Food consumption:** No significant changes were observed in test substance-treated groups, while cyclophosphamide-treated animals showed a reduction in food consumption compared to untreated controls, with a maximum of -22.32% on study day 21 (Table 5).

TABLE 5. Average daily food consumption (gm±SD) of mice during 28 days of treatment[a]

Treatment Group	Week 1	Week 2	Week 3	Week 4
Control (0 ppm) (n=8)	4.2±0.6	5.3±1.5	5.1±1.3	5.5±0.8
Low (50 ppm) (n=8)	5.3±2.2	4.4±0.5	4.5±0.5	4.3±0.4
Mid (125 ppm) (n=8)	4.5±0.6	4.7±0.6	4.9±0.7	4.6±0.6
High (250 ppm) (n=8)	4.4±0.5	4.8±0.6	5.0±0.7	4.7±0.6
Cyclophosphamide 10 mg/kg/day (n=8)	5.0±2.0	4.4±07	3.9±0.9	4.1±0.5

[a] Data obtained from pages 50 & 51, MRID # 48233701.

2. **Water consumption:** No test-related changes were observed, no numerical data were provided.

3. **Compound consumption:** (time-weighted average). The study author provided only the means of the three treatment groups without Standard Deviation. Also the mean compound consumption for the cyclophosphamide-treated animals was not provided (Table 6).

TABLE 6. Mean daily compound consumption (mg/kg bw/day) of mice during 28 days of treatment[a]

Treatment Group	Week 1	Week 2	Week 3	Week 4
Control (0 ppm) (n=8)	0	0	0	0
Low (50 ppm) (n=8)	12.8	10.1	9.9	12.5
Mid (125 ppm) (n=8)	27.6	27	25	26.7
High (250 ppm) (n=8)	52.3	54.1	53.3	52.2

[a] Data obtained from pages 56, MRID # 48233701.

4. **Food efficiency:** Not calculated

D. GROSS NECROPSY: All gross lesions observed in saflufenacil-treated animals occurred individually, and were considered to be non-treatment related. One animal in the high dose group showed minimal thoracic effusion, pericardial deposition as well as a gastric ulcer with

discoloration of gastrointestinal content. These lesions were also considered to be spontaneous and unrelated to treatment.

1. **Organ weight:** When compared to control group, both absolute and relative liver weights of the highest dose level were significantly increased by 16% and 12 %, respectively. Cyclophosphamide- treated animals showed significant decrease of terminal body weights, thymus, and spleen weights (relative weight not significant) (Table 7).

TABLE 7. Terminal body and organ weights of mice treated with to saflufenacil for 28 days [a]

Treatment Group	Body Weight		Liver Weight		Spleen Weight		Thymus Weight	
	Absolute (gm)	Relative (100% control)	Absolute (mg)	Relative (100% control)	Absolute (mg)	Relative (100% control)	Absolute (mg)	Relative (100% control)
Control (0 ppm)	19.6±1.4	100	902±93	4.6±0.2	56±12	0.29±0.04	26±4	0.13±0.03
Low (50 ppm)	19.6±1.2	100	890±61	4.5±0.2	54±5	0.28±0.02	27±5	0.14±0.03
Mid (125 ppm)	19.3±0.7	100	933±129	4.8±0.7	57±10	029±0.05	29±2	0.15±0.01
High (250 ppm)	20.3±1.1	100	1044±102	5.2±0.6*	56±10	0.27±0.36	29±3	0.14±0.02
CP	17.6±1.6*	100	810±113	4.6±0.3	44±8*	0.25±0.06	16±6**	0.09±0.05*

[a] Data obtained from pages 63-66, MRID # 48233701.
*Statistically different from vehicle control ($p < 0.05$).
* Statistically different from vehicle control ($p < 0.01$).
CP: Cyclophosphamide

2. **Histology**: Histopathological evaluation of the liver revealed a slight to moderate centrilobular fatty change in almost all animals (7 out of 8) of the high dose group. Additionally, two animals showed minimal lymphoid infiltration and a single animal showed minimal extramedullary hematopoiesis in the liver.

E. CLINICAL PATHOLOGY:

1. **Hematology:** Red blood cell (RBC) counts, hemoglobin and hematocrit values were decreased in both mid and high dose groups. Cyclophosphamide-treated mice showed decreased RBC, hemoglobin and hematocrit values, while mean corpuscular volume (MCV) and the mean corpuscular hemoglobin content (MCH) were increased (Table 8)

TABLE 8. Hematological values of mice exposed to saflufenacil for 28 days [a]

Treatment Group	RBCs 12^{10}/L	HB mmol/L	HCT L/L	MCV fl	MCH fmol	MCHC mmol/L
Control (0 ppm)	10.4±0.5	9.4±0.4	0.47±0.017	45.2±1	0.90.01	20±0.38
Low (50 ppm)	10.3±0.5	9.1±0.4	0.46±0.018	44.9±0.9	0.89±0.01	19.8±0.4
Mid (125 ppm)	9.4±0.8**	8.4±0.7**	0.421±0.3**	45±1	0.89±0.02	19.9±0.3
High (250 ppm)	9.2±0.6**	8.1±0.4**	0.41±0.25**	44.6±1	0.89±0.02	19.9±0.5
CP	8.8±0.6**	8.3±0.5**	0.42±0.26**	47.1±0.5**	0.94±0.01**	20±0.25

[a] Data obtained from pages 57 & 58, MRID # 48233701.
*Statistically different from vehicle control ($p < 0.05$).

* Statistically different from vehicle control ($p < 0.01$).
RBCs: Erythrocytes, HB: Hemoglobin, HCT: Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration
CP: Cyclophosphamide.

2. **Clinical Chemistry:** Alanine aminotransferase (ALT) activity was increased in the high dose group, while the alkaline phosphatase (ALP) activity was lower in the mid, high, and positive control groups compared to controls. However, ALP decreases were not dose-dependent and was regarded as incidental (Table 9).

TABLE 9. Serum chemistry values of mice exposed to saflufenacil for 28 days [a]

Treatment Group	ALT µkat/L	AST µkat/L	ALP µkat/L
Control (0 ppm)	1.13±0.14	7.52±1.49	2.13±0.15
Low (50 ppm)	0.95±0.19	8.24±1.31	2.04±0.35
Mid (125 ppm)	1.41± 0.73	7.62±2.01	1.55±0.3**
High (250 ppm)	2.92±1.32*	8.12±1.47	1.66±0.16**
CP	1.35±0.98	10.85±5.55	1.81±0.57**

[a] Data obtained from pages 59 & 60, MRID # 48233701.
*Statistically different from vehicle control ($p < 0.05$).
* Statistically different from vehicle control ($p < 0.01$).
ALT: alanine aminotransferase, AST: Aspartate aminotransferase, ALP: Alkaline phosphatase.
CP: Cyclophosphamide.

F. IMMUNOTOXICITY TESTS:

a. **Enzyme-linked immunosorbent assay (ELISA):** Six days after immunization, no significant changes in the SRBC IgM titers were observed in mice dosed with saflufenacil. Evaluation of individual animal data of the treatment and control groups did not show any trend or distribution that would demonstrate a significant suppression of anti-SRBC IgM response. Positive control group demonstrated significant suppression of anti-SRBC IgM response compared with the control group (Table 10).

TABLE 10. Results of anti-SRBC IgM concentrations of mice exposed to saflufenacil for 28 days [a]

Treatment Group	SRBC LU/mL
Control (0 ppm)	3631±1636
Low (50 ppm)	3459±1003
Mid (125 ppm)	3624±1480
High (250 ppm)	3795±1652
CP	479±223**

[a] Data obtained from pages 61, MRID # 48233701.
*Statistically different from vehicle control ($p < 0.01$).
CP: Cyclophosphamide.

b. **NK cells activity assay**: Not performed.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS:

The investigators concluded that under the conditions of the study, BAS 800 H (Saflufenacil) did not reveal any signs of immunotoxicity when administered via the diet over a period of 4 weeks to male C57BL/6J Rj mice. Cyclophosphamide administration (10 mg/kg body weight/day) resulted in immunotoxicity characterized by lower SRBC IgM antibody titres as well as reduced spleen and thymus weights.

B. REVIEWER COMMENTS:

In an immunotoxicity study (MRID # 48233701), Saflufenacil (93.8% a.i., batch # COD-000515) was administered to 8 male C57BL/6J Rj mice in diet at dose levels of 0, 50, 125, or 250 ppm (0, 10, 27, or 52 mg/kg/day) over a period of 4 weeks. Additional 8 male C57BL/6 J Rj mice received the immune-suppressant cyclophosphamide monohydrate at a dose level of 10 mg/kg/day by gavage for 4 weeks (positive control group). The male mouse is the appropriate species/sex for this study. Parameters evaluated were food consumption and body weight (once a week), signs of toxicity or mortality at least once a day. Detailed clinical examinations in an open field were conducted prior to the start of the administration period and weekly thereafter. Measurement of the anti-SRBC IgM antibody titers were performed at the end of the study. Additionally, all animals were assessed by gross pathology; weights of liver, spleen and thymus were measured. In addition, the livers of all animals in test groups (0 and 250 ppm) were examined histopathologically. Limited hematological and clinical chemistry parameters were evaluated too.

On Day 23, all animals were immunized intraperitoneally with 0.5 mL sheep red blood cells (4×10^8 SRBC/mL). On Day 29, the immunotoxic potential of saflufenacil was assessed through the evaluation of the T-cell dependent antibody response (TDAR) to sheep red blood cells (SRBC) using an enzyme-linked immunsorbent assay (ELISA).

Saflufenacil did not cause mortality or any signs of general in any treated group; body weight, body weight changes, food consumption were not affected by treatments. Absolute and relative liver weights of the highest dose were significantly increased by 16% and 12 %, respectively and alanine aminotransferase (ALT) activity was increased. Histopathological evaluation of the liver revealed a slight to moderate centrilobular fatty change in almost all animals (7 out of 8) of the high dose group. Additionally, two animals in the high dose group showed minimal lymphoid infiltration and a single animal showed minimal extramedullary hematopoiesis in the liver. Red blood cell (RBC) counts, hemoglobin and hematocrit values were decreased in both mid and high dose groups. Cyclophosphamide- treated animals showed significant decrease of terminal body weights, thymus, and spleen (relative weight not significant) and decreased RBC, hemoglobin and hematocrit values, while mean corpuscular volume (MCV) and the mean corpuscular hemoglobin content (MCH) were increased.

The systemic NOAEL is 125 ppm (27 mg/kg/day), the LOAEL was 250 ppm (52 mg/kg/day) based on increased liver weights, ALT level and histopathologic findings in the liver.

There were no treatment-related effects in the anti-SRBC IgM titers in mice dosed with saflufenacil. Evaluation of individual animal data for the treatment and control groups did not show any trend or distribution that would demonstrate significant suppression of anti-SRBC IgM

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response. Positive control group demonstrated significant suppression of anti-SRBC IgM response compared with the control.

The NK cells activity assay was not performed. Evaluation of toxicity database of saflufenacil identified the hematopoietic system as the target organ. Increased spleen weights were seen in rat study which was attributable to an increased clearance of defective RBCs (i.e, defective hemoglobin synthesis) and is thus an indication of toxicity to the hematopoietic system rather than to the immune system. The overall weight of evidence suggests that this chemical does not directly target the immune system. Under the HED guidance, if the TDAR assay is negative and evaluation of observational endpoints from all available toxicology database provide no evidence of immunotoxicity, the test article is considered no evidence of immunotoxicity and evaluation of NK cells activity is not necessary.

Under conditions of this study, the NOAEL for anti-SRBC IgM response is 250 ppm (52 mg/kg body weight/day). The LOAEL was not established.

This immunotoxicity study is classified acceptable/guideline and satisfies the guideline requirement for an immunotoxicity study (OPPTS 870.7800) in rats.

C. STUDY DEFICIENCIES:

- No NK cell activity assay was performed. Justification for not requiring a NK cell activity assay was provided by the reviewer.

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Chemical Name:	**Benzamide, 2-chloro-5-[3,6-dihydro-3-methyl-2,6-dioxo-4-(trifluoromethyl)-1(2H)-pyrimidinyl]-4-fluoro-N-[[methyl(1-methylethyl)amino]sulfonyl]-**
PC Code:	**118203**
HED File Code:	**13000 Tox Reviews**
Memo Date:	**3/6/2011**
File ID:	**00000000**
Accession #:	**000-00-0137**

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